Effects of Thyroxin on Amino Acid Incorporation into Protein

Abstract. The effect of thyroxin on the in vitro incorporation of DL-leucine-1-C¹⁶ into the protein of rat liver homogenates has been investigated. Both thyroxin pretreatment in vivo and thyroxin in vitro at a concentration of $1\times 10^{-8}M$ were found to increase the rate of amino acid incorporation. The increased activity following the thyroxin pretreatment in vivo was found to be localized in the mitochondrial fraction. It is suggested that the acceleration of metabolic rate characteristic of thyroxin action may be secondary to the stimulation of energy-requiring reactions such as protein synthesis.

Recent concepts of the mechanism of action of thyroxin have emphasized its uncoupling effect on oxidative phosphorylation (1, 2). This effect, however, is observed only with relatively high concentrations of thyroxin, occurs equally well with both the p- and L- forms (1), and, except for changes in oxidative metabolism, explains few of the physiological effects of the thyroid hormone. Many clinical features of thyroid disease suggest a major, if not primary, role of the thyroid hormone in protein metabolism. In immature animals it is involved in growth; in adults it causes pronounced changes in nitrogen metabolism. Furthermore, in the adult brain and testisorgans in which the quantities of protein and lipid turned over per unit time are apparently negligible compared with turnover of carbohydrate, as evidenced by a respiratory quotient of approximately 1 (3)—the characteristic acceleration of metabolic rate that is observed in almost all other tissues is absent in hyperthyroidism (4).

Previous observations (5) have indicated that thyroxin pretreatment in vivo stimulates amino acid uptake into the protein of rat liver slices. To investigate further the apparent relationship between thyroid function and protein synthesis, studies were undertaken to determine the effects of in vivo and in vitro thyroxin administration on the in vitro incorporation of pL-leucine-1-C14 into the proteins of rat liver homogenates. Livers from 90- to 150-g fasting, male Sprague-Dawley rats were homogenized by means of glass homogenizers in 5 ml of 0.25M sucrose solution per gram of tissue. Homogenization was performed at 0° to 2°C, and tissue fractions were maintained at that temperature through all subsequent operations until final incubation. Intact cells, nuclei, and cell debris were removed by centrifugation at 700g for 10 minutes. The supernatant fluid was spun at 54,000g for 60 minutes in a

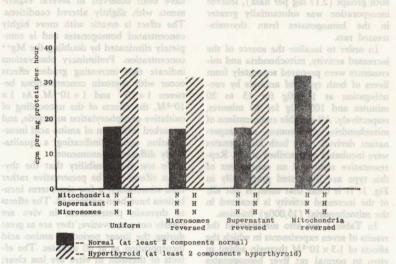


Fig. 1. Localization of increased amino acid incorporating activity in fractions of liver homogenates from rats pretreated with thyroxin. Results are representative of seven such experiments.

Spinco model L ultracentrifuge. The sediment, containing both mitochondrial and microsomal fractions, was suspended in appropriate amounts of 0.25M sucrose and supernatant fluid to yield a suspension containing particulate fractions and supernatant fluid equivalent to approximately 200 mg and 30 mg of liver, respectively, per 0.45 ml, the quantity of homogenate added to each of the experimental flasks.

In eight experiments rats were paired for age and weight; one received almost daily intraperitoneal injections of 100 µg of sodium thyroxin in 1 ml of 0.01N NaOH; the other received equivalent amounts of the NaOH solution alone. After at least six doses in 7 days, homogenates were prepared simultaneously from both animals as described above, and pL-leucine-1-C14 incorporation activity in both was measured in parallel flasks in a single combined experiment. Flask contents and incubation procedure are described in the title of Table 1. The reaction was terminated with 12-percent trichloroacetic acid, and the precipitated protein was purified and plated on filter paper by a modification of the method of Siekevitz (6). Sample weights were determined from difference in planchet weights before and after plating. Radioactivity was measured with a thin-window Geiger-Mueller counter; total counts collected were sufficient to yield a 3-percent coefficient of variation. Counting rates were corrected for background, self-absorption, and zero time controls. The results are summarized in Table 1. Although protein nitrogen concentrations, as determined by the micro-Kjeldahl technique, were identical in

Table 1. Effects of thyroxin on DL-leucine-1-C¹⁴ incorporation into protein of rat-liver homogenates. To each flask (25-ml Erlenmeyer) were added 5 µmole of adenosine-5'-monophosphate, 20 µmole of potassium phosphate (pH 7.4), 5 µmole of MgCl₂, 50 μmole of potassium α-keto-glutarate, 0.8 μmole of DL-leucine-1-C¹⁴ (specific activity, 5.33 µc/µmole), and 0.45 ml of the appropriate homogenate prepared in 0.25M sucrose, as described in the text. In in vitro studies, 0.022 umole of sodium thyroxin contained in 0.1 ml of 0.01N NaOH was added to the experimental flasks; all other flasks received equivalent amounts of the NaOH solution alone. The reaction mixture was brought to a final volume of 1.7 ml with 0.25M sucrose. Incubation in air was carried out with shaking in a water bath at 37°C for 1 hour. Zero time controls were included in all experiments.

Item	Activity (count/min mg of protein per hr)	
	Mean	Standard
Thyroxin pro		in vivo
	rat pairs) 29.0	+10
Normal rat		± 1.9
Hyperthyroid rat	42.3	± 3.0
Difference	13.3*	± 3.2
Effect (%)	+46	
Treatment with in vitro (1.3 × 10-5	
Control	26.9	± 1.8
Thyroxin-treated	31.9	± 2.3
Difference	5.0*	±1.4
Effect (%)	+ 19	

^{*} Denotes statistical significance; p < .02 (determined by method of paired comparison).

both groups (2.17 mg per flask), leucine incorporation was substantially greater in the homogenates from thyroxintreated rats.

In order to localize the source of the increased activity, mitochondria and microsomes were prepared separately from livers of both types of animals by centrifugation at 15,000g for 15 to 20 minutes and 105,000g for 60 minutes, respectively. All possible combinations of mitochondria, microsomes, and supernatant derived from both homogenates were incubated as described above. Representative results of an experiment of this type are illustrated graphically in Fig. 1. It is clear that most, if not all, of the increased activity is localized in the mitochondrial (15,000g) fraction.

In Table 1 are also summarized the results of seven experiments in which the effects of $1.3 \times 10^{-5}M$ thyroxin added in vitro to normal rat liver homogenates were studied under the conditions specified. Although less pronounced, the effects were just as consistent as those observed with thyroxin administration in vivo, a stimulation occurring in every one of the experiments. Similar effects have been observed in several experiments with slightly altered conditions. The effect is erratic with more highly concentrated homogenates and is completely climinated by doubling the Mg++ concentration. Preliminary observations indicate that increasing graded effects occur with thyroxin concentrations between 1×10^{-7} and $1 \times 10^{-4}M$. At $1 \times$ 10-3M, the effects of the uncoupling of oxidative phosphorylation supersede, and a marked inhibition of amino acid incorporation occurs, indicating a qualitatively different phenomenon.

To test the possibility that the thyroxin effects may be preservative rather than stimulatory, a few short-term incubations have been performed. The effects of thyroxin pretreatment in vivo are clearly not preservative; they are as great during the linear period of amino acid incorporation as at 60 minutes. The effects of thyroxin in vitro are less clear; they are distinctly present during the linear period but become more pronounced with longer incubation.

The results of these studies (7) suggest that uncoupling of oxidative phosphorylation is not a physiological action of thyroxin. They support, rather, the hypothesis that thyroxin stimulates energy-requiring processes, such as protein synthesis, and that its characteristic acceleration of oxygen consumption is secondary to the increased demand.

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References and Notes

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